# Correlation between polymorphism in the inosine triphosphatase and the reductions in hemoglobin concentration and ribavirin dose during sofosbuvir and ribavirin therapy

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# Correlation between polymorphism in the inosine triphosphatase and the reductions in hemoglobin concentration and ribavirin dose during sofosbuvir and ribavirin therapy

**Short running title**: *ITPA* polymorphism and SR therapy

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**Acknowledgements**: This study was supported in part by Japan Society for the Promotion of Science Grant 30291595 (A.T.). We are grateful to Ms. Yoko Yasuhara, Ms. Tomoko Hirano, and Ms. Sanae Deguchi for collecting data.

**Conflicts of interest**: In the past year, Dr. Akihiro Tamori has received research funding from Chugai Pharmaceutical and Dr. Norifumi Kawada has received research funding from MSD K.K. and Gilead Sciences. The remaining authors who took part in this study declare that they have nothing to disclose regarding funding or conflicts of interest with regard to this manuscript.

**Abbreviations**: Hb, hemoglobin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; *IL28B*, interleukin 28B, *ITPA*, inosine triphosphatase; PEG-IFN, pegylated interferon-α; SNP, single-nucleotide polymorphism; SVR, sustained virological response

**Abstract**

**Background and Aim**: It is unclear whether polymorphism in the inosine triphosphatase (*ITPA*) gene correlates with the reduction in hemoglobin (Hb) concentrations during sofosbuvir (SOF) and ribavirin (RBV) therapy. We investigated the effects of the *ITPA* polymorphism in Japanese patients with chronic hepatitis C virus genotype 2 infection treated with SOF/RBV therapy.

**Methods**: In 106 patients treated with SOF/RBV therapy, we assessed the effects of the *ITPA* polymorphism (rs1127354) on anemia, RBV dose reduction, and sustained virological response (SVR).

**Results**: Of the 106 patients, 80 had the CC genotype, whereas 26 had a non-CC genotype in *ITPA*. Patients with the CC genotype had significantly larger reductions in Hb concentrations than those with a non-CC genotype throughout the treatment course. RBV dose reduction was required in 18/106 (17.0%) patients, with a significantly higher frequency in patients with the CC genotype than in those with a non-CC genotype ($p = 0.010$). In multivariate analysis, age $\geq$ 65 years ($p = 0.011$) and the *ITPA* CC genotype ($p < 0.0001$) were factors significantly associated with anemia throughout the treatment course. SVR was achieved in 99.0% of all patients: 98.7% of patients with the CC genotype and 100% of patients with a non-CC genotype.

**Conclusions**: *ITPA* polymorphism appeared to correlate with anemia incidence and RBV dose reduction during SOF/RBV therapy, but not the clinical outcome. Careful monitoring of Hb concentrations and prompt adjustment of RBV doses are required for successful treatment, particularly in patients harboring the *ITPA* CC genotype or age $\geq$ 65 years.

**Key words**: hepatitis C virus, hemolytic anemia, inosine triphosphatase, single-nucleotide polymorphism, sustained virological response

## Introduction

Hepatitis C virus (HCV) affects about 170 million people worldwide and is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC).[1] Elimination of HCV is important for preventing HCC, especially in elderly patients with advanced fibrosis.[2,3]

Pegylated interferon-α (PEG-IFN) and ribavirin (RBV) combination therapy was the standard of care for chronic HCV genotype 2 or 3 infection until recently. The rate of sustained virological response (SVR) with this regimen has been reported to be relatively high (~80%).[4–6] However, there are some adverse effects induced by PEG-IFN/RBV therapy. In particular, RBV dose reduction or withdrawal from treatment due to RBV-induced hemolytic anemia was required in 10.0–20.0% of patients treated with PEG-IFN/RBV.[7–11] Moreover, previous studies reported that RBV dose reduction may affect treatment outcomes.[7, 12]

Recently, sofosbuvir (SOF), a nucleotide analog inhibitor of the non-structural protein (NS) 5B polymerase, has been approved for patients with chronic HCV infection. In global and Japanese phase III trials, SOF/RBV combination therapy for patients with chronic HCV genotype 2 infection showed very high SVRs (86.0–98.0%).[13-16] In global phase III trials, 6.0–9.0% and 0.4–1.3% of patients had hemoglobin (Hb) concentrations < 10.0 g/dL and < 8.5 g/dL, respectively, during SOF/RBV combination therapy.[13–15] In a Japanese phase III trial, 12.0% and 1.0% of patients had Hb concentrations < 10.0 g/dL and < 8.5 g/dL, respectively.[16] Similar to the previous PEG-IFN/RBV combination therapy, RBV-induced hemolytic anemia seems to be a common adverse event during SOF/RBV combination therapy.

A genome-wide association study showed a strong association between single-nucleotide polymorphisms (SNPs) at the inosine triphosphatase (*ITPA*) gene on chromosome 20, which causes ITPase deficiency and RBV-induced hemolytic anemia.[17]

In the Caucasian population, there are two reported functional SNPs (rs7270101 and rs1127354) in the *ITPA* gene, which are protective against RBV-induced anemia in patients treated with PEG-IFN/RBV combination therapy in the early weeks of treatment or throughout the treatment course, but they were not associated with clinical outcome.[17–19] However, one of these SNPs (rs7270101) is absent in Japanese patients.[19]

Although polymorphism in the *ITPA* gene correlates with the reduction in Hb concentrations during PEG-IFN/RBV combination therapy, its effects in SOF/RBV therapy have not been reported. Therefore, this study investigated the effects of *ITPA* polymorphism (rs1127354) on anemia throughout the treatment course, RBV dose reduction, and treatment outcome in Japanese patients with chronic HCV genotype 2 infection treated with SOF/RBV combination therapy.

## Patients and Methods

### Patients

This study enrolled 106 patients with chronic HCV genotype 2 infection who were treated with SOF/RBV combination therapy between July 2015 and December 2015 at Osaka City University Hospital. Patients with other hepatitis virus infections, human immunodeficiency virus coinfection, autoimmune liver diseases, alcoholic liver injury, and HCC were excluded.

This study was conducted according to the guidelines of the 1975 Declaration of Helsinki (2004 version). Written informed consent was obtained from all patients prior to treatment. The study protocol was approved by the Ethics Committee of Osaka City University Hospital (no. 3131). The trial was registered with the University Hospital Medical Information Network (no. 000019399).

**Study design**

All patients received SOF/RBV for 12 weeks. SOF (Sovaldi, Gilead Sciences, Tokyo, Japan) was given orally once per day at a dose of 400 mg. RBV (Rebetol, MSD K.K., Tokyo, Japan, or Copegus, Chugai Pharmaceutical, Tokyo, Japan) was given orally twice a day at a total dose of 600–1,000 mg according to body weight. The RBV dose was reduced when Hb concentrations dropped to < 10.0 g/dL, and RBV was discontinued when Hb concentrations dropped to < 8.5 g/dL.

**Routine laboratory examinations**

Blood cell counts and biochemical tests were performed using standard procedures. HCV-RNA was determined using the TaqMan HCV assay (COBAS TaqMan HCV assay; Roche Molecular Diagnostics, Tokyo, Japan) with a lower limit of quantification of 15 IU/mL and an upper limit of quantification of $6.9\times10^7$ IU/mL (1.2–7.8 log IU/mL). The HCV genotype was determined using a HCV genotype primer kit (Institute of Immunology, Tokyo, Japan).

**Study endpoints**

The primary endpoint was anemia, defined as an Hb concentration < 10.0 g/dL or a reduction in Hb concentration by > 2.0 g/dL from baseline and RBV dose reduction throughout the treatment. A secondary endpoint was $SVR_{12}$. $SVR_{12}$ was defined as undetectable HCV RNA in serum at the end of treatment and 12 weeks post-treatment. A relapse was defined as undetectable HCV RNA at the end of treatment and reappearance of HCV RNA within 12 weeks after the end of treatment. Virological responses included the proportion of patients with rapid virological response (RVR; undetectable serum HCV-RNA at week 4), and end-of-treatment response (ETR; undetectable serum

HCV-RNA at the end of treatment). All methods of assessing treatment efficacy were defined according to existing guidelines.[20] During the follow-up period, clinical, biochemical, and quantitative serum HCV-RNA assessments were evaluated at 1–3-month intervals. Safety evaluations included adverse event reporting, laboratory test values, physical examinations, and vital sign assessments.

**SNP genotyping**

We examined genetic polymorphisms in the interleukin 28B (*IL28B*) and *ITPA* genes in patients who consented to genetic analyses.[21, 22] Whole blood was collected from patients and centrifuged to separate the buffy coat. Genomic DNA was extracted from the buffy coat using a QIAamp DNA Blood Midi Kit (QIAGEN, Maryland, USA). Genetic polymorphisms *IL28B* rs8099917 and *ITPA* rs1127354 were genotyped using the TaqMan SNP genotyping Assay with the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All samples were also genotyped by direct sequencing. The primers and procedures used were previously described.[22] The non-TT genotypes, including heterozygosity (TG) and homozygosity (GG) for the minor allele (G), were defined as the *IL28B* minor type, whereas homozygosity for the major allele (TT) was defined as the *IL28B* major type. The non-CC genotype, including heterozygosity (CA) and homozygosity (AA) for the minor allele (A), was defined as the *ITPA* minor type, whereas homozygosity for the major allele (CC) was defined as the *ITPA* major type.

**Statistical analysis**

All statistical analyses were conducted using JMP software (ver. 12.0; SAS Institute, Cary, NC, USA). Continuous variables were compared between groups using the Mann-Whitney U-test, and discontinuous variables were compared using the $\chi^2$ test and Fisher's exact test.

Kaplan-Meier analysis and the log-rank test were used to estimate and compare RBV dose reductions between the groups. Variables with p values < 0.05 in univariate analyses were subjected to step-wise multivariate logistic regression analysis. In the two-tailed test, p values < 0.05 were considered to indicate statistical significance.

## Results

### Baseline patient characteristics

Baseline patient characteristics are shown in Table 1. Of the 106 patients, 80 patients had the CC genotype, whereas 26 patients had a non-CC genotype in the *ITPA* gene at rs1127354. There was no significant difference in baseline characteristics between CC and non-CC genotype groups. The baseline median Hb concentration was 13.5 (range, 10.7–17.6) g/dL and 14.3 (10.0–16.8) g/dL in the CC and non-CC genotype groups, respectively.

Of the 106 patients, 27 had low Hb concentrations (< 12.0 g/dL) at baseline and started with a RBV dose reduced by 200 mg. Of these, 21 patients had the CC genotype and six had non-CC genotypes in the *ITPA* gene.

### Association between *ITPA*-SNP and reduction in Hb concentrations during SOF/RBV combination therapy

Figure 1 shows the time-dependent reduction in Hb concentration during SOF/RBV combination therapy. Patients with the CC genotype showed significantly larger reductions in Hb concentration than those with non-CC genotypes throughout the treatment course. The greatest differences in the reduction of mean Hb concentration were found at weeks 2, 4, and 6 ($p < 0.0001$).

Additionally, a reduction of Hb concentration by > 2.0 g/dL from baseline was observed more frequently in the CC genotype group than in the non-CC genotype group throughout the treatment course (Fig. 2, 3B). The difference between the groups was most pronounced at week 4 ($p < 0.0001$).

Throughout the treatment course, 14.2% (15/106) of patients had Hb concentrations < 10.0 g/dL and no patient (0/106) had Hb concentrations < 8.5 g/dL. As shown in Figure 3A, no patient with a non-CC genotype had Hb concentrations < 10.0 g/dL at week 4 or throughout the treatment. By contrast, 7.5% (6/80) and 18.8% (15/80) of patients with the CC genotype had Hb concentrations < 10.0 g/dL at week 4 ($p = 0.17$) and throughout the treatment, respectively ($p = 0.010$).

**Association between *ITPA*-SNP and RBV dose reduction during SOF/RBV combination therapy**

RBV dose reduction was required in 18 patients with the CC genotype (17.0%), but not in those with non-CC genotypes throughout the treatment course. The difference in the number of weeks until the first RBV dose reduction by *ITPA*-SNP was determined with the log-rank test (Fig. 4). The frequency of RBV dose reduction was significantly higher in patients with the CC genotype than in those with non-CC genotypes ($p = 0.010$).

By contrast, the total RBV dose during 12 weeks was comparable between the patients with the CC genotype (median total dose 50.200 mg) and those with non-CC genotypes (median total dose 50.200 mg; $p = 0.12$; Supplementary Fig. 1). The total RBV dose per body weight was also comparable between the groups (data not shown).

**Predictive factors associated with anemia throughout the treatment course during SOF/RBV combination therapy**

Anemia was seen in 31 patients (29.2%) and in 56 patients (52.8%) at week 4 and throughout the treatment course, respectively. Of patients with the CC genotype, 39.2% exhibited anemia at week 4, but none of those with a non-CC genotype did so ($p < 0.0001$; Fig. 3C). Moreover, the incidence of anemia throughout the treatment course was higher in patients with the CC genotype (66.3%) than in those with non-CC genotypes (11.5%; $p < 0.0001$).

Univariate analysis showed that predictive factors associated with anemia throughout the treatment course were age ≥ 65 years ($p = 0.0044$; OR, 3.177; 95% CI, 1.435–7.036), baseline serum creatinine ≥ 0.7 mg/dL ($p = 0.039$; OR, 2.286; 95% CI, 1.043–5.009), and the TT genotype in the *IL28B* gene ($p =0.046$; OR, 2.928; 95% CI, 1.018–8.422), as well as the CC genotype in the *ITPA* gene ($p < 0.0001$; OR, 15.049; 95% CI, 4.146–54.632; Table 2).

Supplementary Fig. 2 shows the numbers of patients with and without anemia throughout the treatment course for each potential predictor, namely, *ITPA* genotype, age, baseline serum creatinine, and *IL-28B* genotype.

In the multivariate analysis, age ≥ 65 years ($p = 0.011$; OR, 3.432; 95% CI, 1.325–8.890) and the CC genotype in the *ITPA* gene ($p < 0.0001$; OR, 18.380; 95% CI, 4.503–75.027) were factors significantly associated with anemia throughout the treatment course.

**Association between *ITPA*-SNP and treatment outcome with SOF/RBV combination therapy**

One patient was withdrawn from combination therapy at week 3 due to rejection of the treatment, and six patients were lost to follow-up after the end of treatment. Treatment was not discontinued in any patient because of adverse events.

The RVR, ETR, and $SVR_4$ were 92.0%, 100%, and 99.0%, respectively. $SVR_{12}$ was achieved in 99% of patients: 98.7% of patients with the CC genotype and 100% of patients with a non-CC genotype (Supplementary Fig. 3). Thus, $SVR_{12}$ was similar between CC and non-CC genotype groups ($p = 0.76$). A patient with the CC genotype in the *ITPA* gene relapsed at week 4 after treatment. S282 resistance-associated variants in NS5B were not detected at baseline or post-treatment in this patient.

## Discussion

To our knowledge, this is the first report demonstrating an association between *ITPA* polymorphisms and RBV-induced hemolytic anemia with SOF/RBV therapy for patients with chronic HCV genotype 2 infection in the real world. Our results suggest that the *ITPA* polymorphism correlates with anemia incidence and RBV dose reduction, but not the clinical outcome, during SOF/RBV therapy.

A serious adverse event in PEG-IFN/RBV combination therapy is RBV-induced hemolytic anemia, which leads to RBV dose modification in ~10.0–20.0% of patients.[7–11] In the phase III NEUTRINO trial, 23.0% and 2.0% of patients had Hb concentrations < 10.0 g/dL and < 8.5 g/dL, respectively, during SOF/PEG-IFN/RBV triple therapy.[13] Thus, it is important to monitor Hb concentrations carefully, and to adjust the RBV dose promptly for successful treatment.

Although hemolytic anemia is milder in SOF/RBV therapy than in IFN-based regimens, previous studies of SOF/RBV combination therapy suggested that the rates of anemia in a Japanese trial tended to be higher than those in global trials. In fact, our results indicated that 14.2% and 0% of patients had Hb concentrations < 10.0 g/dL and < 8.5 g/dL, respectively, consistent with the Japanese clinical trial. This difference may be caused by

the fact that HCV-infected older patients with advanced liver fibrosis have been included in Japan,[12] and Japanese patients with chronic HCV infection were more than 10 years older than those in Western countries.[23] In fact, the mean age of the enrolled patients was around 50 years old in the global phase III trials, whereas it was 57 years old in the Japanese phase III trial. In our study, it was 63 years old, and, in particular, 52.8% of patients were aged ≥ 65 years. In the present study, the CC genotype in the *ITPA* gene ($p < 0.0001$) and age ≥ 65 years ($p = 0.011$) were independent predictive factors for anemia throughout the treatment course by multivariate analysis (Table 2). Thus, the *ITPA* polymorphism will become increasingly important not only in the Japanese population of elderly patients, but also in Western populations, in the near future.

Previously, Fellay *et al*. reported that non-CC variants in the *ITPA* gene protected against RBV-induced hemolytic anemia, not only in the early weeks of treatment,[17, 19] but also throughout the treatment course.[18] Our study of SOF/RBV was consistent with their findings. Between the two groups, the greatest differences in the reduction of mean Hb concentrations were found at weeks 2, 4, and 6 ($p < 0.0001$), as in treatment with PEG-IFN/RBV. The reductions in mean Hb concentrations at week 4 were −1.82±0.98 g/dL and −0.37±0.79 g/dl in the CC and non-CC genotype groups, respectively (Fig. 1). By contrast, Sakamoto *et al*.[24] reported that the reductions at week 4 during PEG-IFN/RBV combination therapy were −2.9±1.3 g/dL and −1.1±0.7 g/dL in CC and non-CC genotype groups, respectively. Moreover, Suzuki *et al*.[25] reported that reductions at week 4 in telaprevir/PEG-IFN/RBV triple therapy were −3.5±1.1 g/dL and −2.2±0.96 g/dL in CC and non-CC genotype groups, respectively. Thus, the reduction in Hb concentration tended to be milder with SOF/RBV, compared with those in previous studies using PEG-IFN/RBV with or without telaprevir. In addition, patients with non-CC genotype had much lower reductions in Hb concentrations than those in previous studies of

PEG-IFN/RBV combination therapy. A possible explanation for this is that the myelosuppressive action of PEG-IFN contributes to the reduction in Hb concentration during PEG-IFN/RBV combination therapy.

McHutchison *et al*. reported that the administration of < 80.0% of planned RBV was associated with poor treatment outcomes with PEG-IFN/RBV.[7] This may reflect decreased treatment efficacy due to RBV dose reduction in patients showing severe anemia. However, most studies have reported that the *ITPA* polymorphism was not directly associated with the treatment outcome of PEG-IFN/RBV combination therapy for patients with chronic HCV genotype 1 [17–19, 24, 26–28] and genotype 2/3 infection.[29]

In this study of SOF/RBV, the administration of < 80.0% of planned RBV did not result in a decrease of SVR ($p = 0.32$). Additionally, no significant association between the *ITPA* polymorphism and treatment outcome by SOF/RBV combination therapy was apparent (Supplementary Fig. 3). Thus, our results suggest that, although the *ITPA* polymorphism influenced RBV dose reduction during SOF/RBV combination therapy, RBV dose reduction was not significantly associated with SVR.

In this study, 18/106 (17.0%) patients required RBV dose reduction. However, no patients discontinued RBV treatment because the Hb concentration did not drop to < 8.5 g/dL in any of them. We suggest that completion of RBV administration during the scheduled treatment period is important in achieving SVR, even if accompanied by RBV dose reduction.

Kurosaki *et al*.[27] reported that the non-CC genotype of the *ITPA* gene was associated with a high rate of SVR with PEG-IFN/RBV combination therapy in patients with the TT genotype in the *IL-28B* gene. However, our study indicated that the $SVR_{12}$ with SOF/RBV combination therapy was similar in the CC and non-CC genotype groups in patients with the TT genotype in the *IL-28B* gene: 100% of patients with the CC

genotype and 100% of patients with a non-CC genotype.

RBV is a synthetic guanosine nucleoside analog that inhibits the replication of various RNA and DNA viruses, although the exact mechanism of RBV action remains unknown. Although RBV monotherapy has minimal antiviral effects on HCV eradication, previous studies reported that IFN/RBV combination therapy has higher treatment responses than IFN monotherapy.[30] Furthermore, SOF/RBV combination therapy was shown to have a very strong antiviral effect on HCV. However, the rate of SVR during SOF monotherapy was reported to be very low (60.0%) when the treatment regimen did not include RBV in the phase II ELECTRON trial.[31] Thus, SOF-based therapy is recommended in combination with RBV, indicating that RBV remains a key drug for the treatment of chronic HCV genotype 2 infection. Additionally, for most IFN-free therapies, it is recommended to include a RBV-containing regimen for patients with cirrhosis.[32–34] Our findings showing the effect of *ITPA* polymorphism on the reduction of hemoglobin levels and RBV dose during SOF/RBV combination therapy may be applicable to treatment with other RBV-containing regimens.

There are reports that patients with the *ITPA*-CC genotype had a smaller decrease in the platelet count during PEG-IFN/RBV combination therapy.[35] By contrast, our study indicated that the changes in platelet counts were comparable between the patients with the CC genotype and those with non-CC genotypes during SOF/RBV combination therapy (Supplementary Fig. 4).

Our study had some important limitations. First, this was a single-center study on a relatively small scale. Second, it consisted only of Japanese patients, and we do not know whether the results can be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, our results will promote future studies involving treatment with other RBV-containing regimens on a larger scale and in patients of various ethnicities.

In conclusion, the *ITPA* polymorphism (rs1127354) correlated with anemia incidence and RBV dose reduction during SOF/RBV combination therapy, but not the clinical outcome, in patients with HCV genotype 2 infection. Careful monitoring of the Hb concentration and prompt adjustment of RBV doses are required for successful treatment, particularly for patients harboring the *ITPA* CC genotype or age ≥ 65 years.

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**Table 1**. Baseline characteristics of patients

| | Total (n = 106) | *ITPA* -SNP rs1127354 CC genotype (n = 80) | *ITPA* -SNP rs1127354 non-CC genotype (n = 26) | p-value |
|---|---|---|---|---|
| Age (years) | 66 (32-87) | 67 (32-83) | 62 (35-87) | 0.75 |
| Sex (male/female) | 60/46 | 45/35 | 15/11 | 0.54 |
| Body weight (kg) | 59.3 (36.0-102.7) | 59.0 (36.0-102.7) | 61.1 (41.2-87.0) | 0.76 |
| Previous IFN-based treatment (+/–) | 37/69 | 28/52 | 9/17 | 0.58 |
| HCC history (+/–) | 7/99 | 6/74 | 1/25 | 0.45 |
| *IL28B* -SNP rs8099917 (TT/non-TT) | 87/19 | 67/13 | 20/6 | 0.30 |
| Liver cirrhosis (+/–) | 11/95 | 10/70 | 1/25 | 0.19 |
| White blood cells (/μl) | 4,900 (1,700-9,700) | 4,900 (1,700-8,600) | 5,200 (2,900-9,700) | 0.30 |
| Hemoglobin (g/dl) | 13.8 (10.0-17.6) | 13.5 (10.7-17.6) | 14.3 (10.0-16.8) | 0.37 |
| Platelet count ($\times 10^4/mm^3$) | 16.9 (4.9-33.6) | 16.8 (4.9-26.3) | 17.1 (8.9-33.6) | 0.28 |
| Aspartate aminotransferase (U/L) | 40 (15-200) | 42 (15-200) | 35 (15-141) | 0.60 |
| Alanine aminotransferase (U/L) | 39 (10-297) | 39 (10-297) | 39 (10-104) | 0.59 |
| γ-glutamyltransferase (U/L) | 37 (11-311) | 34 (11-311) | 41 (11-202) | 0.73 |
| Total bilirubin (mg/dl) | 0.7 (0.2-2.4) | 0.7 (0.2-1.9) | 0.6 (0.3-2.4) | 0.38 |
| Creatinine (mg/dl) | 0.74 (0.43-1.12) | 0.75 (0.43-1.12) | 0.70 (0.50-1.10) | 0.82 |
| Estimated glemerular filtration rate ($ml/min/1.73m^2$) | 76.0 (48.5-111.4) | 75.5 (49.1-111.4) | 77.5 (48.5-110.6) | 0.63 |
| α-fetoprotein (ng/ml) | 4.3 (< 2.0-68.8) | 4.2 (< 2.0-68.8) | 4.8 (< 2.0-28.2) | 0.97 |
| HCV-RNA (log IU/ml) | 6.2 (< 1.2-7.3) | 6.1 (< 1.2-7.1) | 6.3 (3.5-7.3) | 0.54 |
| Viral genotype (2a/2b) | 65/41 | 51/29 | 14/12 | 0.25 |

Values are medians (range). IFN, interferon; HCC, hepatocellular carcinoma; *IL28B*, interleukin 28B; SNP, single-nucleotide polymorphism; HCV, hepatitis C virus.

**Table 2**. Predictive factors associated with anemia throughout the treatment course during SOF/RBV combination therapy

| Factor | Category | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | OR | 95 % CI | p-value | | OR | 95 % CI | p-value | |
| Age (years) | ≥ 65 | 3.177 | 1.435-7.036 | 0.0044 | * | 3.432 | 1.325-8.890 | 0.011 | * |
| Sex | male | 1.667 | 0.768-3.616 | 0.20 | | | | | |
| Body weight (kg) | ≥ 55 | 1.529 | 0.691-3.381 | 0.29 | | | | | |
| Previous IFN-based treatment | (+) | 0.654 | 0.293-1.459 | 0.30 | | | | | |
| HCC history | (+) | 1.205 | 0.256-5.667 | 0.81 | | | | | |
| *IL28B*-SNP rs8099917 | TT genotype | 2.928 | 1.018-8.422 | 0.046 | * | 3.167 | 0.927-10.816 | 0.066 | |
| *ITPA*-SNP rs1127354 | CC genotype | 15.049 | 4.146-54.632 | <0.0001 | * | 18.380 | 4.503-75.027 | <0.0001 | * |
| Liver cirrhosis | (+) | 2.611 | 0.653-10.447 | 0.17 | | | | | |
| White blood cells (/μl) | ≥ 5,000 | 1.367 | 0.636-2.940 | 0.42 | | | | | |
| Hemoglobin (g/dl) | ≥ 13.5 | 1.950 | 0.895-4.249 | 0.093 | | | | | |
| Platelet count ($\times 10^4/mm^3$) | ≥ 17.5 | 0.704 | 0.324-1.530 | 0.38 | | | | | |
| Aspartate aminotransferase (U/L) | ≥ 45 | 0.947 | 0.440-2.037 | 0.89 | | | | | |
| Alanine aminotransferase (U/L) | ≥ 45 | 1.286 | 0.596-2.772 | 0.52 | | | | | |
| γ-glutamyltransferase (U/L) | ≥ 25 | 1.294 | 0.581-2.881 | 0.53 | | | | | |
| Total bilirubin (mg/dl) | ≥ 0.7 | 1.074 | 0.501-2.303 | 0.85 | | | | | |
| Creatinine (mg/dl) | ≥ 0.7 | 2.286 | 1.043-5.009 | 0.039 | * | 2.433 | 0.936-6.324 | 0.068 | |
| Estimated glemerular filtration rate (mL/min/1.73m2) | ≥ 75 | 0.494 | 0.227-1.075 | 0.075 | | | | | |
| α-fetoprotein (ng/ml) | ≥ 4.0 | 1.554 | 0.721-3.353 | 0.26 | | | | | |
| HCV-RNA (log IU/ml) | ≥ 6.0 | 1.056 | 0.491-2.272 | 0.89 | | | | | |
| Viral genotype | 2a | 2.118 | 0.956-4.690 | 0.064 | | | | | |

*$p < 0.05$, which was taken to indicate statistical significance. IFN, interferon; HCC, hepatocellular carcinoma; *IL28B*, interleukin 28B; SNP, single-nucleotide polymorphism; *ITPA*, inosine triphosphatase; HCV, hepatitis C virus.

**Figure Legends**

**Figure 1**. Reduction in Hb concentrations during 12 weeks of SOF/RBV treatment with regard to *ITPA*-SNP rs1127354. Of 106 patients with chronic hepatitis C, 80 had the CC genotype (dashed line), whereas 26 had a non-CC genotype (solid line) in the *ITPA* gene.

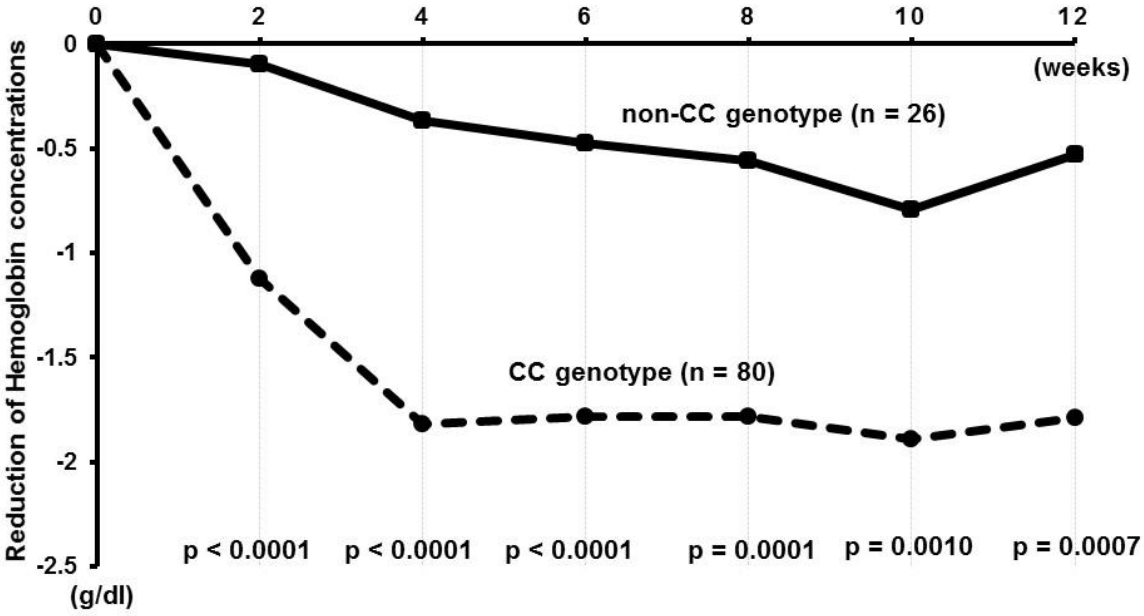



***Figure 1***

**Figure 2**. Proportion of the patients who had a reduction in Hb concentrations of > 2.0 g/dL from baseline with respect to *ITPA*-SNP.

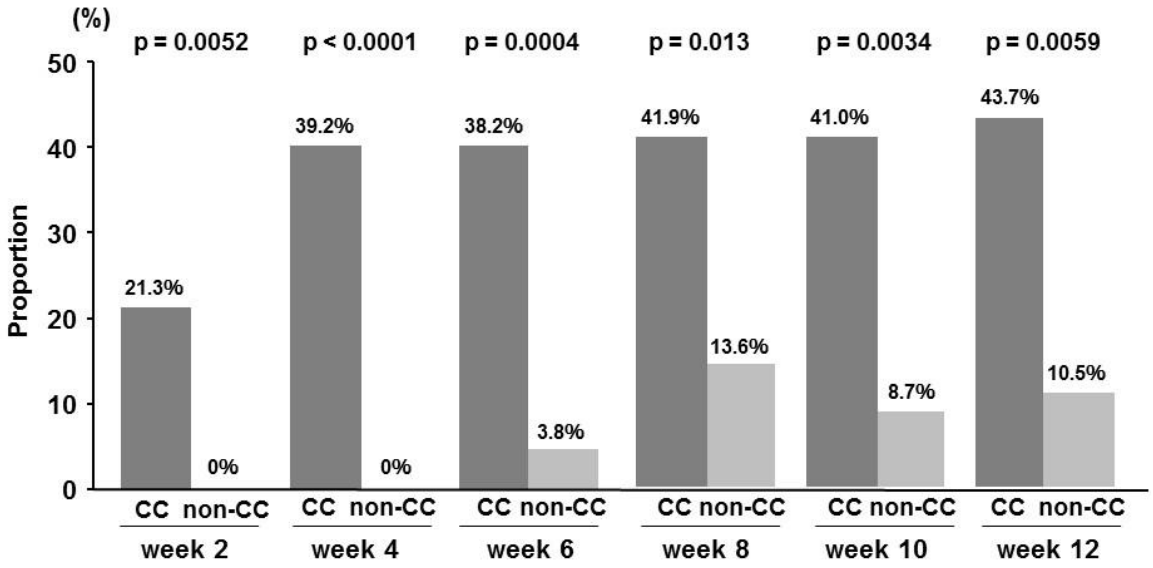



***Figure 2***

**Figure 3**. Proportion of the patients with (A) Hb concentrations < 10.0 g/dL, (B) reduction in Hb concentrations of > 2.0 g/dL from baseline, and (C) either at week 4 or throughout the treatment course, with respect to *ITPA*-SNP.

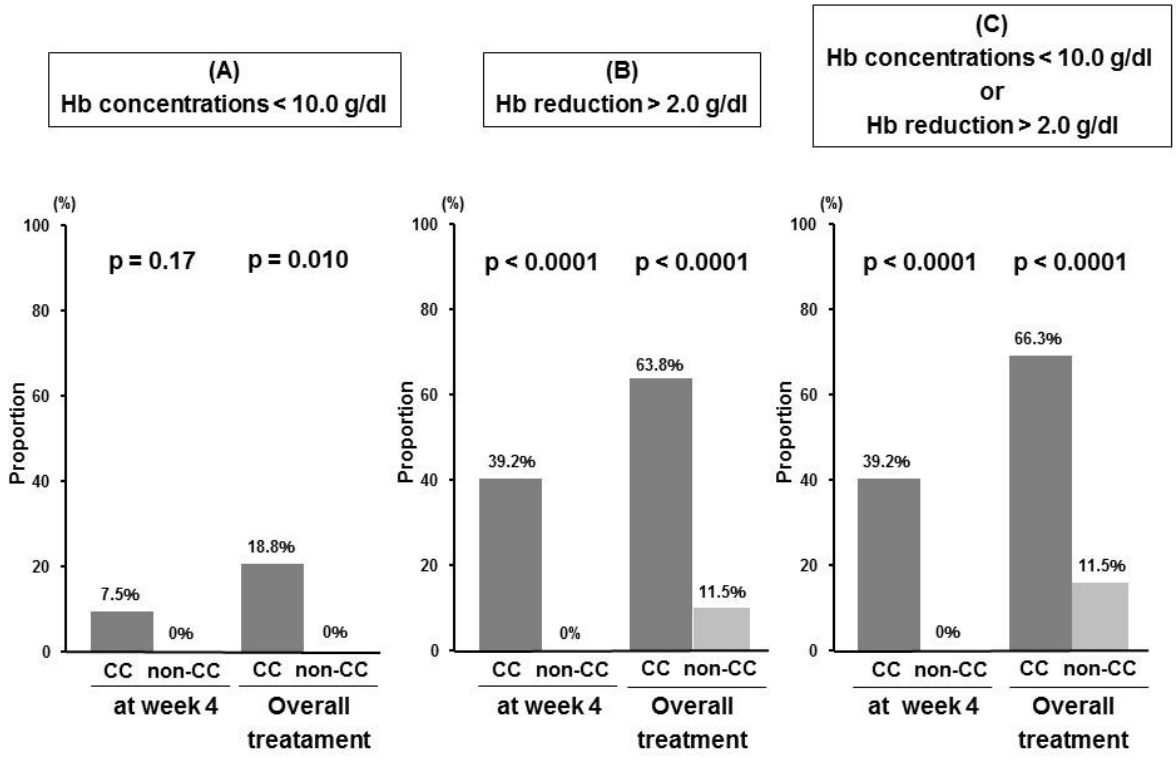



***Figure 3***

**Figure 4**. Kaplan-Meier curve for RBV dose reduction grouped by *ITPA*-SNP rs1127354 (dashed line represents CC genotype; solid line represents non-CC genotype).

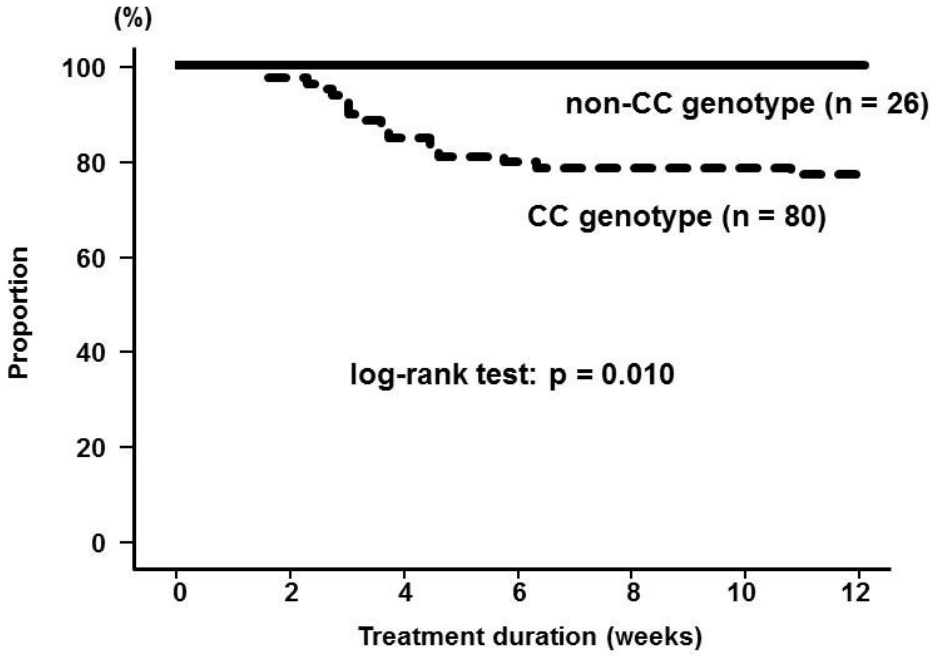
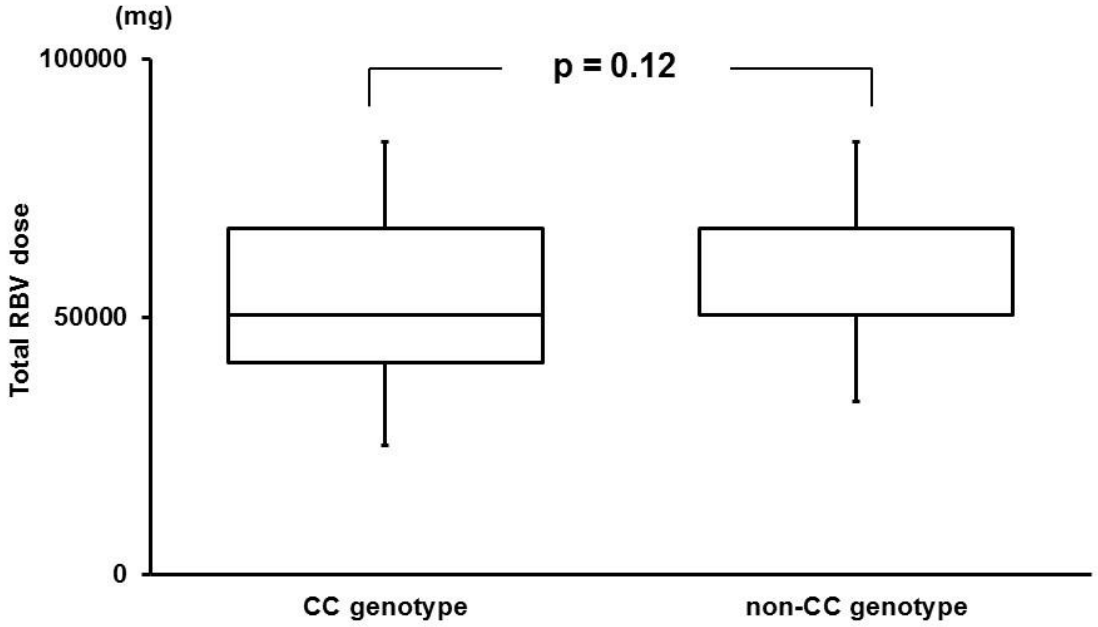



***Figure 4***

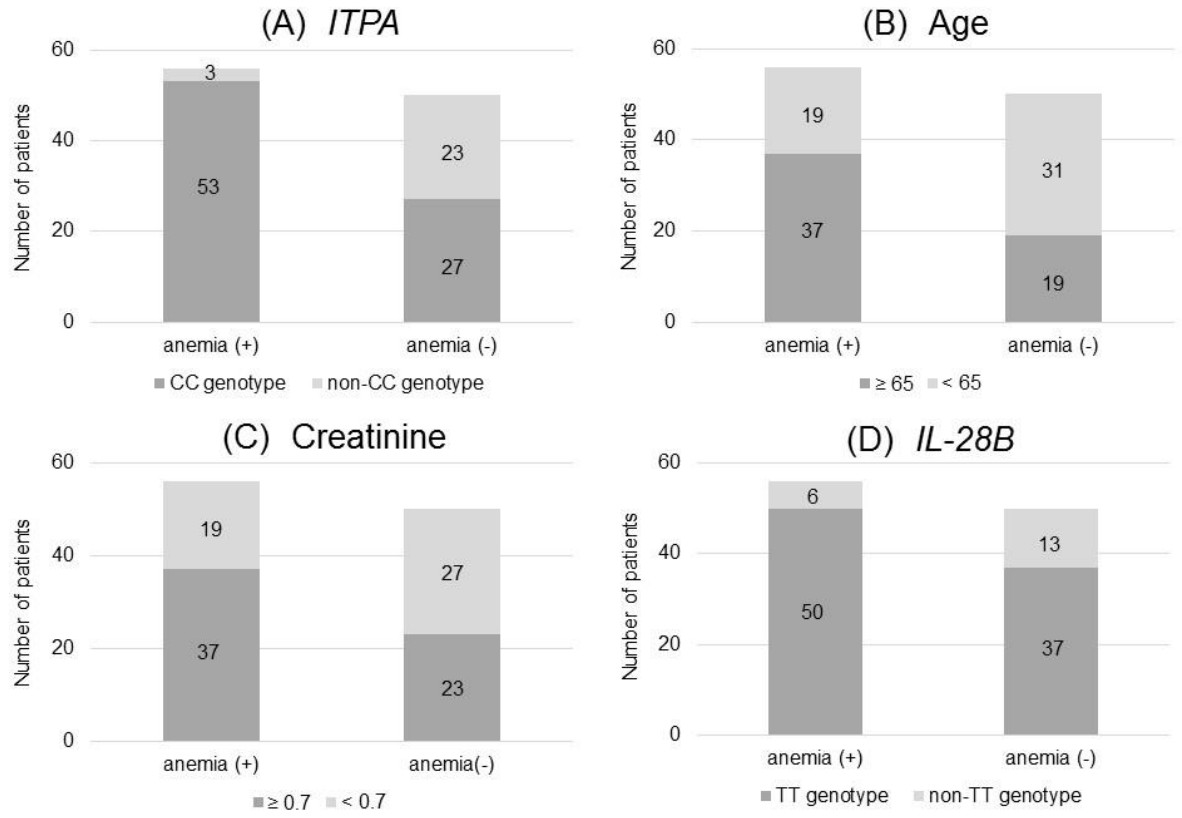
**Supplementary Figure Legends**

**Supplementary Figure 1**. Differences in the total RBV dose during 12 weeks of SOF and RBV treatment with respect to *ITPA*-SNP rs1127354. Medians are shown as horizontal bars. Boxes cover the interquartile range and tails show minimum and maximum values.



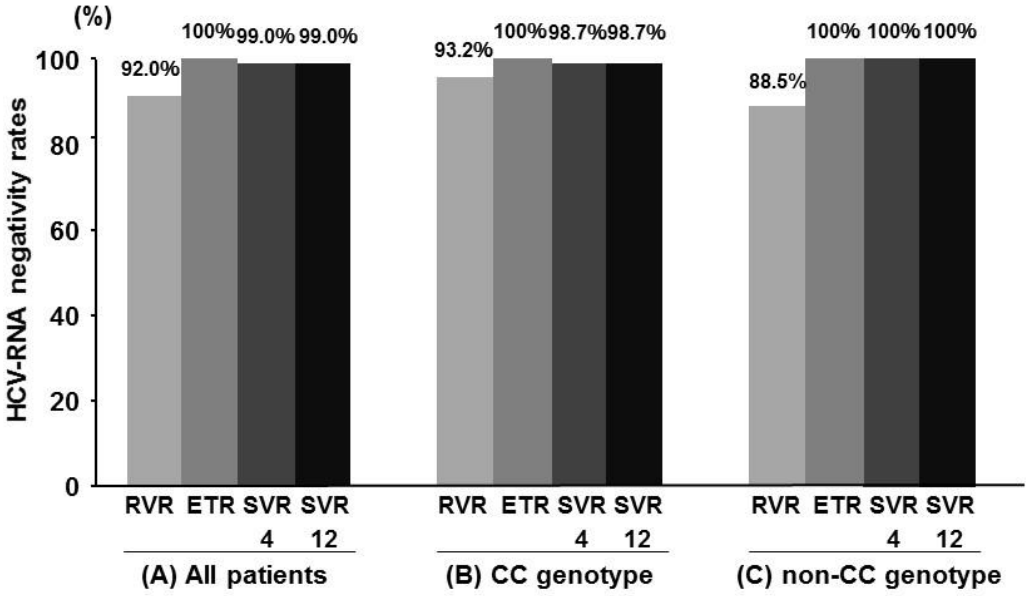

***Supplementary Figure 1***

**Supplementary Figure 2**. The numbers of patients with and without anemia throughout the treatment course for (**A**) *ITPA* genotype, (**B**) age, (**C**) baseline serum creatinine, and (D) *IL-28B* genotype.



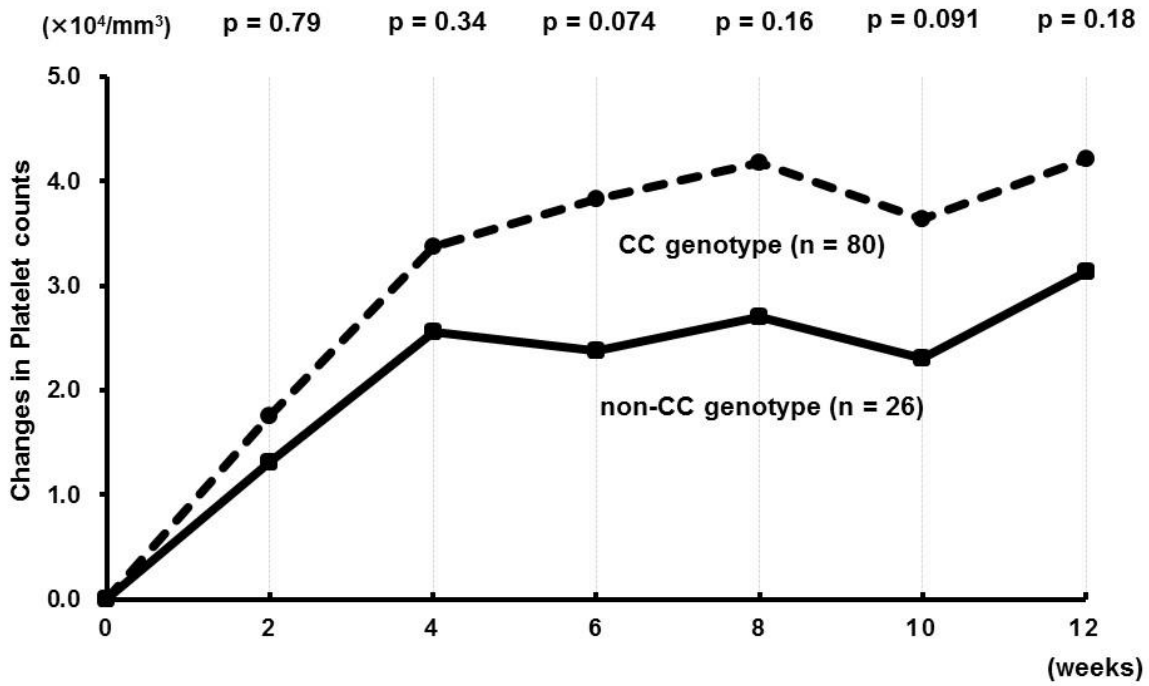

***Supplementary Figure 2***

**Supplementary Figure 3**. Rates of RVR, ETR, $SVR_4$, and $SVR_{12}$ to SOF/RBV treatment among (**A**) all patients, (**B**) patients with the *ITPA* CC genotype, and (**C**) patients with a non-CC genotype.




***Supplementary Figure 3***

**Supplementary Figure 4**. Changes in platelet counts during 12 weeks of SOF/RBV treatment with regard to *ITPA*-SNP rs1127354. Of 106 patients with chronic hepatitis C, 80 had the CC genotype (dashed line), whereas 26 had a non-CC genotype (solid line) in the *ITPA* gene.




***Supplementary Figure 4***